A COMPARATIVE EVALUATION OF THE REMINERALIZING POTENTIAL OF A PROBIOTIC TOOTH PASTE, TRADITIONAL YOGHURT AND A NOVAMIN CONTAINING TOOTH PASTE ON POST - ORTHODONTIC WHITE SPOT LESIONS USING SCANNING ELECTRON MICROSCOPE AND ENERGY DISPERSIVE X- RAY ANALYSIS - AN IN VITRO STUDY.

Dissertation submitted to

THE TAMILNADU DR. M.G.R MEDICAL UNIVERSITY

In partial fulfillment for the degree of

MASTER OF DENTAL SURGERY

BRANCH – V

ORTHODONTICS AND DENTOFACIAL ORTHOPEDICS

2016-2019

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation titled "**A COMPARATIVE EVALUATION OF THE REMINERALIZING POTENTIAL OF A PROBIOTIC TOOTH PASTE, TRADITIONAL YOGHURT AND A NOVAMIN CONTAINING TOOTH PASTE ON POST- ORTHODONTIC WHITE SPOT LESIONS USING SCANNING ELECTRON MICROSCOPE AND ENERGY DISPERSIVE X- RAY ANALYSIS- AN IN VITRO STUDY**" is a bonafide work done by **Dr. R MAIVIZHI,** Postgraduate student, during the course of the study for the degree of **MASTER OF DENTAL SURGERY** in the specialty of **DEPARTMENT OF ORTHODONTICS AND DENTOFACIAL ORTHOPAEDICS,** Vivekanandha Dental College for Women, Tiruchengode, during the period of 2016-2019.

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ENDORSEMENT BY THE HEAD OF THE DEPARTMENT AND HEAD OF THE INSTITUTION

This is to certify that **Dr. R MAIVIZHI**, Post graduate student (2016-2019) in the **DEPARTMENT OF ORTHODONTICS AND DENTOFACIAL ORTHOPAEDICS,** Vivekanandha Dental College for Women, has done this dissertation titled, "**A COMPARATIVE EVALUATION OF THE REMINERALIZING POTENTIAL OF A PROBIOTIC TOOTH PASTE, TRADITIONAL YOGHURT AND A NOVAMIN CONTAINING TOOTH PASTE ON POST- ORTHODONTIC WHITE SPOT LESIONS USING SCANNING ELECTRON MICROSCOPE AND ENERGY DISPERSIVE X- RAY ANALYSIS- AN IN VITRO STUDY**" under our guidance and supervision in partial fulfillment of the regulations laid down by the Tamilnadu Dr. M.G.R Medical University, Chennai – 600032 for M.D.S **Branch – V**

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DECLARATION

TITLE OF DISSERTATION	A comparative evaluation of the remineralizing potential of a probiotic tooth paste, traditional yoghurt and a novamin containing tooth paste on post-orthodontic white spot lesions using scanning electron microscope and energy dispersive x- ray analysis- an in vitro study.
PLACE OF STUDY	Elayampalayam, Tiruchengode, Namakkal District
DURATION OF THE COURSE	3 Years (2016-2019)
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I hereby declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission of the Principal, Vivekanandha Dental College for Women, Tiruchengode. In addition, I declare that no part of this work will be published either in print or in electronic format without the guide who has been actively involved in the dissertation. The author has the right to reserve publishing of work solely with prior permission of the Principal, Vivekanandha Dental College for Women, Tiruchengode.

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PLAGIARISM CERTIFICATE

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TO WHOMSOEVER IT MAY CONCERN

Principal Investigator: Dr. R MAIVIZHI

Title: A comparative evaluation of the Remineralizing potential of a Probiotic tooth paste, traditional yoghurt and a Novamin containing tooth paste on Post – Orthodontic White Spot Lesions using Scanning Electron Microscope and Energy Dispersive X-ray analysis - An in vitro study.

Institutional ethics committee thank you for your submission for approval of above proposal. It has been taken for discussion in the meeting held on 25.10.16. The committee approves the project and it has no objection on the study being carried out in Vivekanandha Dental College for Women.

You are requested to submit the final report on completion of project. Any case of adverse reaction should be informed to the institutional ethics committee and action will be taken thereafter.

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LIST OF ABBREVIATIONS

- WSL White Spot Lesions
- CPP-ACP Casein Phosphopeptide amorphous calcium phosphate
- CPP Casein Phosphopeptide
- SEM Scanning Electron Microscope
- EDAX Energy Dispersive X-Ray Analysis

ANNEXURES

URKUND

Urkund Analysis Result

Analysed Document: Whole MD Compilation.docx (D45657570)
Submitted: 12/13/2018 7:02:00 AM
Submitted By: drmaivizhi@gmail.com
Significance: 15 %

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Though the dissertation holds my name, but the process that lead to its completion is accomplished in combination with the dedicated work of other people.

I wish to acknowledge my gratitude and thankfulness to God, for granting me the wisdom, health and strength to undertake this task and enabling me to its completion and to certain people without whom this would not have taken its form.

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Prof. Dr. S Nagalakshmi, my Guide and the Head of the Department, has been my mentor, well-wisher and my supervisor, who nurtured me and always had brought the best out of me. I am immensely indebted for patiently guiding me throughout. Mam has always made herself available to clarify my doubts despite her busy schedule and I consider it as a great opportunity to do my Postgraduation under her guidance.

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INTRODUCTION

White spot lesions are hypocalcified enamel seen around orthodontic brackets which are of prime concern to orthodontists. The term white spot lesions were defined by Fejerskov et al as "the first sign of a carious lesion on enamel that can be detected with the naked eye"[1]. These lesions are the earliest macroscopic evidence of caries. These lesions present with an intact enamel surface with a subsurface demineralization. Saliva contains calcium ions, phosphate ions, buffering agents, fluoride and other substances where an early stage of demineralization is reversed[2]. Prevalence of WSLs in patients after orthodontic treatment ranges from 15%-85%[3-7] with most studies reporting 50%-70%.[8.9]

Prevention of post orthodontic WSLs is done by maintaining a good oral hygiene[10] along with application of fluoride incorporated varnishes,[11]dentrifice,[12,13] adhesives,[14]elastomerics[15]and use of remineralization products such as those containing CPP-ACP (casein phosphopeptide- amorphous calcium phosphate[16,17] and calcium sodium phosphosilicate(Novamin)[18]. The main idea behind using remineralization agents is to have ions directly delivered to the site most needed.

World Health Organization defines Probiotics as "live microorganisms which when administered in adequate amounts, confer benefits to the health of the host"[19]**.** Probiotic strains have been reported to prevent oral diseases, including caries which maintain or restore the natural micro flora against pathogenic invasion[20,21]**.** Probiotics

in the form of lozenges[22] and toothpastes have proven to produce efficient remineralization of these subsurface lesions[23]

Milk and dairy products have also been identified as having anticariogenic activity. In 6 to 11 year olds, milk acted as a protective agent who did not use fluoride, had poor oral hygiene and frequent sugar consumption as reported by Petti et al[24]. Yoghurt, a dairy product, which is commonly consumed as milk, exhibits higher natural CPPs content than that in milk but has been subjected to less research in the context of its oral health, especially on dental enamel. The CPP's have a remarkable ability to bind to amorphous calcium phosphate and stabilize in dental plaque. Studies have shown that acid dissolution of human enamel is reduced by over 50 per cent in vitro[25].A study by Chanchal et al showed that the traditional yoghurt has more inhibitory effect on dental enamel demineralization than that of an probiotic enriched yoghurt[26].

The need of the study is to search for an easily available, effective method in treating WSL which made us to compare the efficacy of Probiotic tooth paste and traditional yoghurt with already validated a Novamin containing tooth paste as a remineralizing agent.

A wide range of both macroscopic and microscopic assessment methods are available at present that are used in-vitro and in-vivo. It is desirable that the

assessment method be accurate, reproducible, easy to use and should validate mineral loss from the enamel. For microscopic in-vitro evaluation, various methods like caries models, polarized light microscopy and Scanning Electron Microscope have been used.

Energy-dispersive X-ray Analysis is an analytical technique used to estimate quantitatively the amounts of mineral in a given tooth sample[27].

Thus, this study was designed to evaluate the remineralization effects of Novamin containing tooth paste, Probiotic tooth paste and traditional Yoghurt on WSL's that occur around the orthodontic brackets using SEM and EDX analysis.

AIM:

The aim of the present study is to compare the remineralization potential of a Probiotic tooth paste and traditional yoghurt with that of a Novamin containing tooth paste.

OBJECTIVE:

1) Assess qualitatively the remineralizing potential of a Probiotic tooth paste, traditional yoghurt and a Novamin containing tooth paste on demineralized enamel by Scanning Electron Microscope.
2) Assess quantitatively the remineralizing potential of a Probiotic tooth paste, traditional yoghurt and a Novamin containing tooth paste on demineralized enamel by comparing the Ca/P ratios using Energy Dispersive X-ray analysis (EDX).
3) To compare the remineralizing potential among a Probiotic tooth paste, traditional yoghurt and a Novamin containing tooth paste.

REVIEW OF LITERATURE:

Literature has been reviewed under the following headings:

- Incidence and prevalence of White Spot Lesions
- Prevention and Management of White Spot lesions
- Remineralizing potential of Novamin containing paste
- Remineralizing potential of Probiotics and Yoghurt

Incidence and Prevalence of White Spot Lesions:

Richer A E, Arida AO, Peters MC, Sohn W in 2011[28] assessed the relationship of incidence of labial incipient caries with various treatment variables and patients with pretreatment and posttreatement photographs of labial surface of teeth. They concluded that incidence of new white spot lesion was 72.9% and new cavitated lesion was 2.9% and the development was not associated with sex, age but they are related to improper oral hygiene and treatment duration.

Eser Tufekci, Julian S Dixon, JC Gunsolley, Steven J Lindauer in 2011[29] determined the prevalence rate of White spot lesions during orthodontic treatment at 6 and 12 months of treatment based on the visual examination method. This study shows a remarkable increase in the number of WSL's, 38% in the first 6 months of treatment and continued to increase at a slower rate to around 46%upto 12 months. This article emphasized on the importance of evaluating the oral hygiene status during the initial

months of treatment that lead to demineralization and emphasizes proper preventive measures to prevent the occurrence of WSL's.

Alessandra Lucchese, Enrico Gherlone in 2013[6] did a cross sectional study on the prevalence of white spot lesions in patients before and during fixed orthodontic treatment on three group of patients; Group 1: 6 months of orthodontic treatment, Group 2: 12 months of orthodontic treatment and Group 0: the control group. The presence of WSL's was evaluated by the Gorelick's scoring system. There were no significant difference between Group 1 and Group 2 in the prevalence of WSL's but it was significantly higher than Group 0 and no significant difference between boys and girls. Mandibular first molar and maxillary lateral incisors were the most affected teeth in all the groups. They have reported significant decalcification occurred at 6 months after bonding which implies the need for preventive measures and early diagnosis of WSL's.

Rafiqul Islam, Mohammed Khurseed Alam in 2016[4] in their study reviewed 29 in vivo study articles which had a mention about white spot lesions as a part of orthodontic treatment and outlined various agents for prevention of white spot lesions. They concluded that fluoride mouthwashes, tooth paste, varnish and bonding agents and use of casein phosphpeptides, amorphouscalciumphosphate can be used as a preventive agent for white spot lesions during orthodontic treatment.

Kristina Lopatiene, Marija Borisovaite, Egle Lapenaite in 2016[5], did a systematic literature review in a need to update the evidence for the prevention of white spot lesions during and after orthodontic treatment using materials which contains fluoride and/ or casein phosphopeptide- amorphous calcium phosphate. They had reviewed 326 articles and only 12 articles fulfilled their inclusion criteria i.e the study should be on humans and the preventive treatment of WSL's should be done with fluroide containing product and / or casein derivatives. They concluded fluoride and casein substitutes are effective but casein- phosphopeptide- amorphous calcium phosphate was more beneficial than fluoride rinse in reducing the white spot lesions.

Maria GraziaCagetti, Stefano Mastroberardino, EgleMilia, FabiaCocco, Peter Lingstrom and Guglielmo Campus in 2013[30], did a systematic review on the effect of probiotics in caries- prevention. According to the CONSORT guidelines 66 RCT's were reviewed, only 26 fulfilled the inclusion criteria and out of those only three studies had caries lesion development as their outcome. In two thirds of the selected papers, the mutans Streptococci counts in saliva and plaque were reduced with the usage of Probiotics in short term. Although the effect of Probiotics seems to be advantageous in the reduction of WSL's but enough RCT's are needed to substantiate the same.

Nandikolla Sagarika, Sundaramoorthy Suchindran, SC Loganathan, Velayutham Gopikrishna in 2012[31] did an invivo assessment of prevalence of white spot lesion in a section of Indian population undergoing fixed orthodontic treatment for a period of 12-15 months on 12-20 years old patients using the visual International Caries Detection and Assessment System II criteria, 180 patients were divided into two groups; Group 1: undergoing orthodontic treatment for a period of 12-15 months, Group2: control group with the need of orthodontic treatment. The results showed a high prevalence rate of 75.6% in Group 1compared to 15.6% in Group 2.

Prevention and Management of White Spot Lesions:

Gorton J and Featherstone JDB in 2003[32] conducted an in-vivo study to assess the inhibition of enamel demineralization when bonding the brackets using fluoride releasing GIC and composite with no fluoride release. 21 patients who require premolar extractions were bonded consecutively with GIC and composite. The teeth were extracted after 4 weeks and subjected to microhardness around brackets. He concluded that the teeth bonded using GIC showed high microhardness implying presence of cariostatic effect in GIC, but there was no significant increase in salivary fluoride level.

Pascotto RC, Navarro MFL, Filho LC, Cury JA in 2004[14] carried out an in-vivo study to evaluate the effect of enamel demineralization around brackets when bonded with resin- modified glass ionomer cement and conventional composite. 14 patients who required therapeutic extraction of premolar were enrolled and divided into 2 groups. After 30 days the premolar teeth were extracted, the extracted tooth was examined by cross sectional surface hardness. They reported that RMGIC bonded teeth showed decreased caries development.

Wilmot DR in 2003[33] conducted an experimental double blind prospective randomized controlled clinical trial to measure the change in size of Post- orthodontic white spot lesions using polarized light images acquired at debond , 12 and 26 weeks when treated with non fluoridemouthrinse/ toothpaste regimen with that of an low fluoride (50ppm) mouthrinse/toothpaste on a sample of 26 patients. The result showed no significance between the groups, but there was a reduction in white spot lesion to one – third in 12 weeks and half the size in 24 weeks in both the groups and thus the other concluded that there is no clinical advantage in using the low fluoride formulations of mouthrinse or toothpaste.

Brochner A in 2010[34] investigated the effect of topical application of 10%CPP-ACP cream on white spot lesion. 60 patients who developed post orthodontic white spot lesion with visible grading of $\geq$ 1 were studied. They were divided into 2

groups. In Group 1 the patients were instructed to apply CPP-ACP containing agent topically on WSL once daily for period of 4 weeks and to the control group 2, to brush with fluoridated tooth paste. After 4 weeks they were evaluated using quantitative light induced laser fluorescence (QLF) and they concluded that CPP-ACP reduced WSL but it was not superior to that of regular fluoride toothpaste.

Remineralising potential of Novamin Containing paste:

Vahid Golpayegani, Sohrabi, Biria and Ansari in 2012[35] compared the remineralizing potential of Novamin containing dentrifice with that of a fluoridated tooth paste by evaluating the surface microhardness by Vicker'smicrohardness test and concluded that Novamin has a higher remineralization potential than that of the fluoridated tooth paste.

Arun et alin 2013[36] evaluated the caries remineralization potential of three dentrifices, GC Tooth Mousse, Clinpro tooth crème and SHY- NM using micro CT and Vicker'smicrohardness test on 45 extracted single rooted permanent mandibular premolar tooth, 15 each in a group for two cycles of remineralizing agent application at one for 15 days and the other for 30 days which were subjected to micro computed tomography to determine the enamel mineral content and microhardness test both at baseline and after treatment. Among the three remineralizing agents, CPP-ACP has better remineralizing potential then the other paste though all three agents significantly increased the Linear Attentuation Coefficient and Vicker's Hardness Number.

Adit Bharat Mehta, Veena Kumari, Rani Jose, Vajiheh Izadikhah in 2014[37] compared the remineralizing potential of bioactive glass containing pasted- SHY-NM with that of CPP- ACP containing paste GC Tooth Mousse on artificially induced initial carious lesion on extracted premolar teeth by surface microhardness test. Readings were taken at baseline and after treatment with paste for 10 days. The bioactive glass group SHY-NM showed more microhardness. Both BAG and CCP-ACP reminerlize early carious lesion but bioactive glass more effectively remineralized the early carious lesion.

Sri SathyaNarayana, Vinoth Kumar Deepa, Shafie Ahamed, Emmanuel Solomon Sathish, Meyappan, Satheesh Kumar et al in 2014[38] compared the remineralizing efficiency of bioactive glass on artificially induced carious lesion with that of a fluoridated and CPP-ACP containing paste by a high resolution scanning electron microscopy and quantitative assessment by energy dispersive X ray spectroscopy. All the remineralizing agents were applied for 10 min for a period of 10 days. The group that was treated with bioactive glass showed an increase in the calcium content and they concluded that bioactive glass can be considered as an effective remineralizing agent.

Iqra Chaudhary, Abhay M Tripathi, Gunjan Yadav, Soanli Sahain 2017[39] compared the remineralizing potential of calcium sodium phosphosilicate paste and CPP-ACP paste with that of an fluoridated tooth paste in early carious lesion using Scanning Electron Microscopy and Energy Dispersive Xray Analysis for structural and elemental analysis respectively. 60 sound premolars were divided randomly into three groups of 20 each and were treated with calcium sodium phosphosilicate, CPP-ACP paste and fluoridated tooth paste for 7 days after a demineralization cycle of 4 days . Based on Calcium Phosphorus Concentration they concluded that Calcium sodium phosphosilicate paste showed highest remineralizing potential when compared to the CPP-ACP and fluoridated tooth paste.

Ayam A Taha, Mangala P Patel, Robert G Hill, Padhraig S Fleming in 2017[40] conducted a systematic review on the effect of bioactive glasses on enamel remineralization. Inclusion parameters for selection of article were the objective and subjective measures of enamel remineralization, harms, including any evidence of damage to the enamel surface, patient satisfaction and in vitro evidence of enamel remineralization using any recognized laboratory techniques. Eleven in- vitro studies were in cluded in the review, which concluded that enamel remineralization improved with bioactive glasses irrespective of the method of application and is capable of enhancing enamel remineralizaiton in various formulations, but a clinical research to confirm their effectives is overdue.

Remineralising potential of Probiotics and Yoghurt:

Ferrazzano, Canitle, Qurto, Ingenito, Chianese, Addeo in 2008[41] did an invitro study to test the ability of natural Casein Phosphopeptides, contained in yoghurt to prevent demineralization and promote remineralization of dental enamel on artificial carious lesion and evaluated it quantitatively by observing the change in weight and calcium titration and qualitatively by Scanning Electron microscope between groupswith and without natural CPP. Statistically significant differences in weight changes and SEM observations confirm the inhibitory effect or demineralization and remineralizationpotential of the natural CPP's contained in yoghurt.

Jubin Jose, Sridevi Padmanabhan, Arun Chitharanjan et al in 2013[23], evaluated and compared the effects of the systemic consumption of probiotic curd and the topical application of probiotic toothpaste on the Streptococcus mutans levels using real time polymerase in the plaque of 60 orthodontic patients who were divided into three groups of which one was the control group for a period of thirty days. Significant reduction in the S mutans concenterations were seen in groups treated with probiotic toothpaste and probiotic curd, although probiotic toothpaste was found to be more effective but it was not statistically significant.

Chanchal Singh, Sulekha Doley et al in 2016[26] did an in- vitro comparative evaluation of the inhibitory effect of Probiotic Enriched and Traditional yoghurt extracts on enamel demineralization, quantitatively by Atomic Absorption Spectrophotometer and qualitatively by confocal laser scanning microscopy on sixty intact human premolars which were divided into three groups of 20 each, of which one was the control and the other two was the treatment group. Atomic Absorption Spectrophotometer and Confocal Laser scanning Microscopy analysis showed statistically significant differences. They concluded that traditional yoghurt has more inhibitory effect than probiotic enriched yoghurt on enamel demineralization.

MATERIALS AND METHODS:

Eighty extracted human premolars got from patients requiring therapeutic extraction for orthodontic treatment constituted the sample. The ethical clearance was obtained from the Institutuional ethical committee, Vivekanandha Dental College for Women No: VDCW/IEC/34/2016. The teeth were rinsed with saline and stored individually in closed pre-labeled plastic containers(Sample Container (30ml) ASTRA Bioscience Ltd.,) which contained distilled water and stored at room temperature (37°C).

Inclusion criteria for teeth being selected were intact buccal enamel without any developmental defects, no physical damage due to extraction, not subjected to pretreatment chemical agents, and any caries or WSLs.

The apical two thirds of the root were sectioned for operational convenience. The teeth were polished with rubber cup and pumice, sprayed with water and dried with a compressed oil-free air stream. The buccal surfaces of the premolars were etched with 37% phosphoric acid gel (ETCH, d- tech, Sakh iChem Tech Pvt. Ltd., India) for 15seconds and the etchant gel was removed with a water spray and dried, followed by bonding of premolar brackets (Gemini Series, .022 MBT brackets, 3M Unitek, Monrovia, USA) using a light cure adhesive (Transbond® XT, 3M Unitek, Monrovia, USA) on the buccal surface. Buccal surface of the bonded teeth were coated with acid-resistant nail varnish (Colorbar cosmetics PvtLmt, USA), leaving a 1 mm wide window around the bracket.

Samples were stored in individual pre-labeled plastic containers containing distilled water at room temperature, and were randomly assigned to four groups, ie. Group 1: Treatment with Novamin containing tooth paste(SHY NM- Group Pharamaceuticals Limited, Batch No: B.SN633) Group 2: Treatment with Probiotic tooth paste (PerioBiotic Probiotic Toothpaste, Designs for Health, Canada, Lot No: #8148) Group 3: Treatment with traditional yoghurt and Group 4: Control group, No treatment- containing 20 premolar teeth until they were subjected to SEM scanning and EDAX analysis for the baseline reading (T0)ie. just after bonding.

After the baseline reading, each tooth was immersed in 10 ml of demineralizing solution for 48 hours to artificially produce caries lesion. The demineralizing solution was made with 2.0mM ($Ca(NO_3)_2.4H_20$, 2.0mM (KH_2PO4), 75mM acetic acid with the pH adjusted to 4.4 according to Featherstone et al[42]. After demineralization, all the samples were subjected to SEM scanning again and evaluated by EDX analysis for (T1) reading ie. after demineralization.

The samples were then given treatment according to Table 1:

Table 1: Distribution of Groups and treatment.

Sample	n	Treatment given
Group 1	20	Novamin containing tooth paste- 2 times daily, every 12 hours, 30 sec manual brushing placed in artificial saliva
Group 2	20	Probiotic tooth paste – 2 times daily, every 12 hours, 30 sec manual brushing, placed in artificial saliva
Group 3	20	Traditional yoghurt, 2 times daily, every 12 hours, 30 sec manual brushing, placed in artificial saliva.
Group 4	20	Control group- No treatment, placed in artificial saliva

The samples were placed in artificial saliva at room temperature. The composition of the artificial saliva is 2.200 g/L gastric mucin, 0.381g/L *NaCl*, 0.213 g/L ($CaCl_2$)$2H_2O$, 0.738 g/L K_2 (HPO_4)$3H_2O$ and 1.114 g/L KCl. The final pH was adjusted to 7.00 at 37°C.[43]

At the end of the 30 days treatment period all the samples were again evaluated by EDX analysis for T2 reading ie. 30 days post treatment. Thus Ca/P ratios were analysed for both the groups at three time intervals:

T0 representing 0 day just after the bonding,

T1 after 48 hours of demineralization and

T2 representing 30days post-treatment.

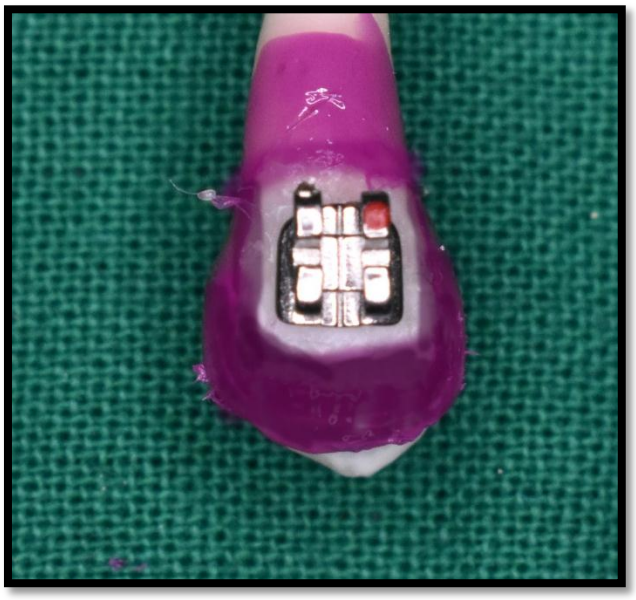
Figure 1: Extracted tooth stored in labelled individual container

Figure 2: d-tech 37%Phosphoric acid

Figure 3: 3M Transbond Bonding Agent.

Figure4: Tansbond XT Adhesive

Figure 5: Brackets bonded on the tooth surface leaving 1mm wide window around the bracket



Reagents of Dimeralizing solution and artificial saliva

Figure 6: Sodium sulphide hydrate

Figure 7: Sodium carbonate anhydrate

Figure 8: Sodium Chloride

Figure 9: Potassium Chloride

Figure 10: Calcium hydrate tetrahydrate

Figure 11: Urea

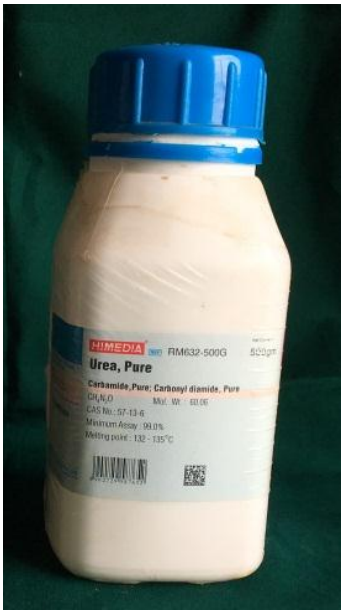


Figure 12: Calcium Chloride Dihydrate

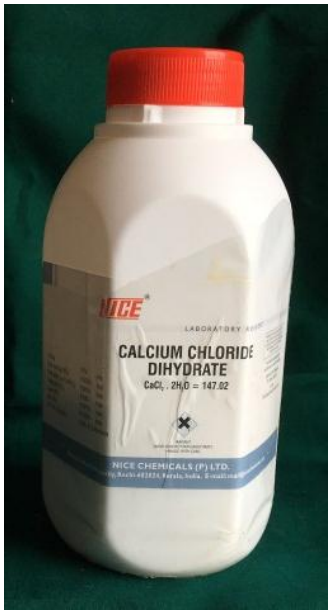


Figure 13: Acetic acid

Figure 14: Disodium phosphate

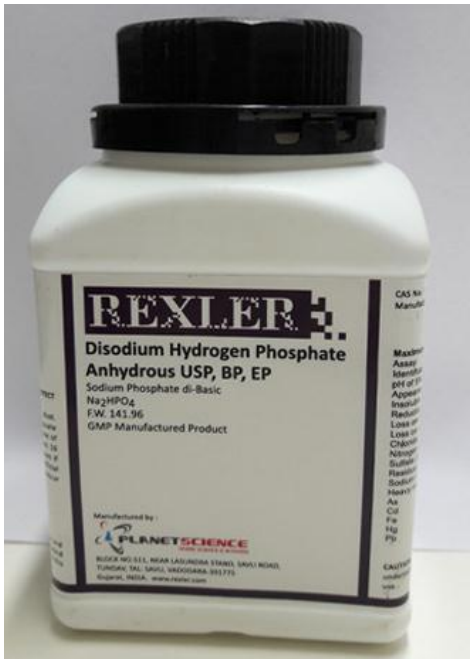


Figure 15: Monopotassium Phosphite

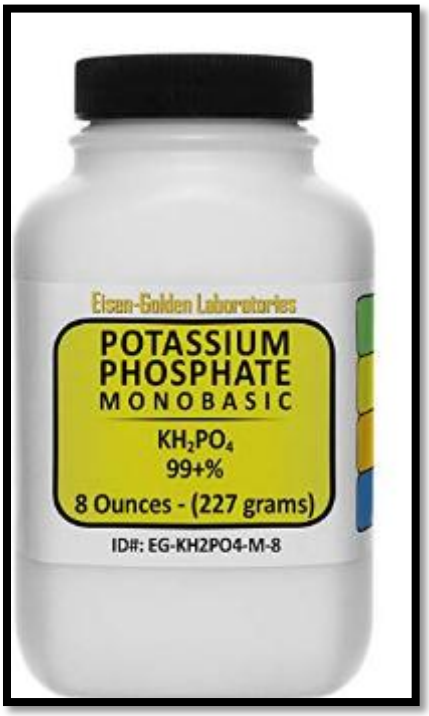


Figure 16: Samples randomly selected, assigned to groups and stored in separate containers

Figure 17: Novamin containing paste and Probiotic tooth paste

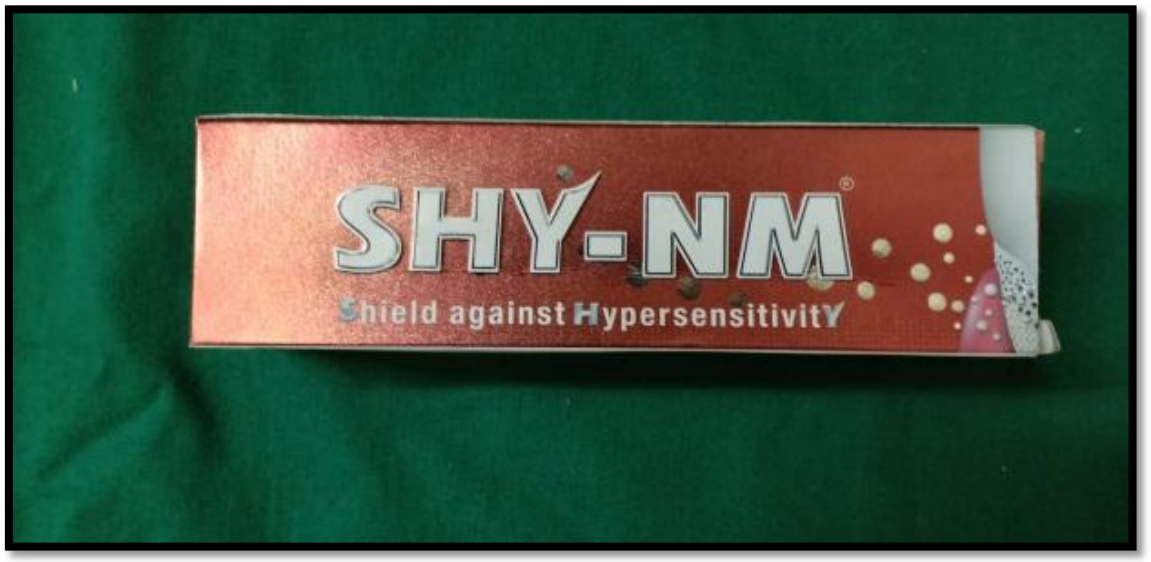
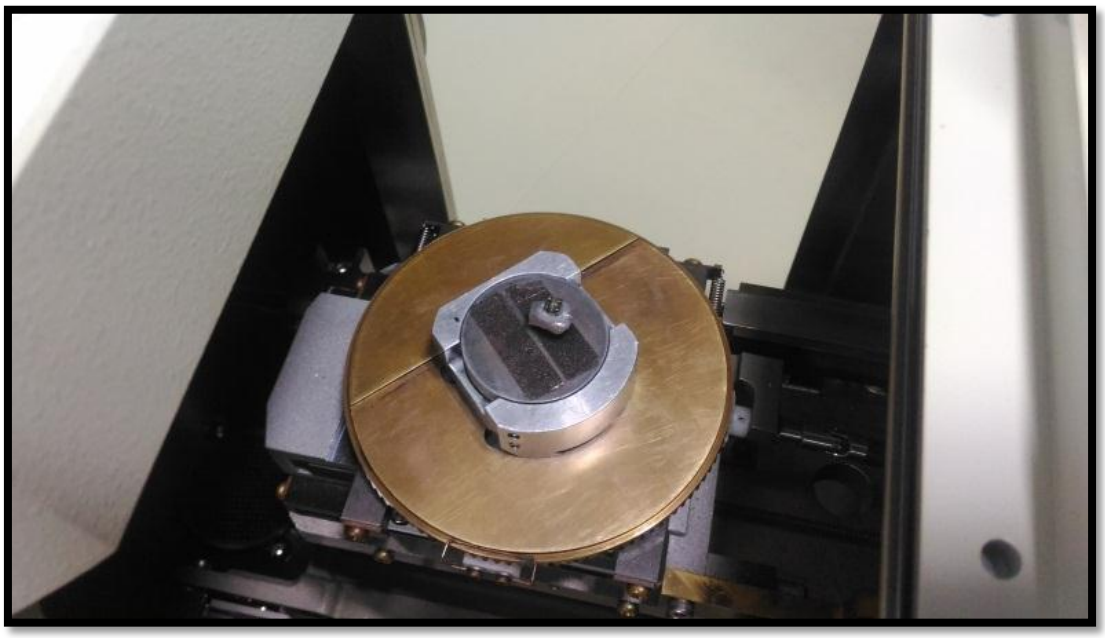


Figure 18: Traditional Yoghurt

Figure 19: Gold sputtering Unit

Figure 20: Energy Dispersive X-ray Analysis machine.

Figure 21: Tooth sample placed inside the unit for SEM and EDAX analysis



STATISTICAL ANALYSIS

Kolmogorov- Smirnov and Shapiro – Wilk tests were used to test normal distribution of the data.

The data was normally distributed. One way analysis of variance (ANOVA) was used to determine statistically significant differencs between the means of four groups 1, 2, 3 and 4.

The formula used for the ANOVA analysis

$$\text{ANOVA} = \frac{\text{BMS- WMS}}{\text{BMS +(n-1) WMS}}$$

Where,

BMS = Between subjects mean sum of squares

WMS = Within subjects mean sum of squares

n = Number of measurements.

Post Hoc tukey test was done for multiple comparisons between groups and to determine which specific group showed significance.

Paired T- test was done to compare at T1, T2 and T3 of each of the groups.

p value of less than 0.05 was considered to be statistically significant.

Samples were grouped in the following order.

Group 1: Novamin treated

Group 2: Probiotic treated

Group 3: Yoghurt treated

Group 4: Control group

RESULTS:

The amount of demineralization and remineralization of the tooth samples in the groups were quantitatively measured by assessing the Ca/P ratio by Energy Dispersive X- Ray Analysis (EDAX) at three time intervals. i.e at

T0- 0 day just after bonding,

T1 – after 48 hours of demineralization and

T2- representing 30 days post-treatment.

The mean values of the Ca/P ratio of the four groups were compared, ie, Novamin, Probiotic, Yoghurt and Control group at three time intervals from T0 through T2 using the SPSS version. Based on Shapiro Wilks test, the data collected was in normal distribution.

T0 is the baseline reading to evaluate the Ca/P ratio of sample teeth prior to demineralization. The mean Ca/P ratio for all the 4 groups was 2.238 ± 0.225.

All samples were subjected to demineralization for 48 hours in the demineralization solution. T1 gives the Ca/P ratio post demineralization. The decrease in the mean value indicates the amount of demineralization that has occurred. At T1, the mean Ca/P ratio was 1.9251 ± 0.0844.

Samples of Group 1, 2, and 3 were subjected to remineralizingagents ,Novamin, Probiotic and Yoghurt respectively and the group 4 – control group was not treated with remineralizing agent but was placed in artificial saliva. There was an increase in the mean value of the ratio of Calcium and Phosphorus in the all the groups.

The pre and post treatment Ca/P ratios (T1 and T2) of all 4 groups were statistically analysed using the Paired T – test. The test revealed that there were significant statistical difference within each group between T1 i.eCa/P ratio of demineralized tooth and T2 ie. after 30days of treatment with the remineralizing agents for all groups except for Group 4 i.e Control Group as in Table 1 indicating that statistically significant amount of remineralisation occurred in all the treated groups.

Table 1:Paired T- test for comparison of time intervals within the group.

Groups		**Mean**	**N**	**Std. Deviation**	**Std. Error**	**Paired mean difference**	**Std. Deviation**	**Std. Error**	**p value**
Group 1	T1	1.9265	20	0.09675	0.2163	-0.2280	.24106	.05390	.000*
	T2	2.1550	20	0.27144	0.6070				
Group 2	T1	1.9230	20	0.7948	0.1777	-0.23400	.21288	.04760	.000*
	T2	2.1570	20	0.18860	0.4217				
Group 3	T1	1.9280	20	0.8733	0.0195	-0.34750	.24303	.05434	.000*
	T2	2.2755	20	0.26255	0.05871				
Group 4	T1	1.9230	20	0.07948	0.0177	-0.05550	.11592	.02592	.045
	T2	1.9785	20	0.10796	0.02414				

*** p value significant when <0.05**

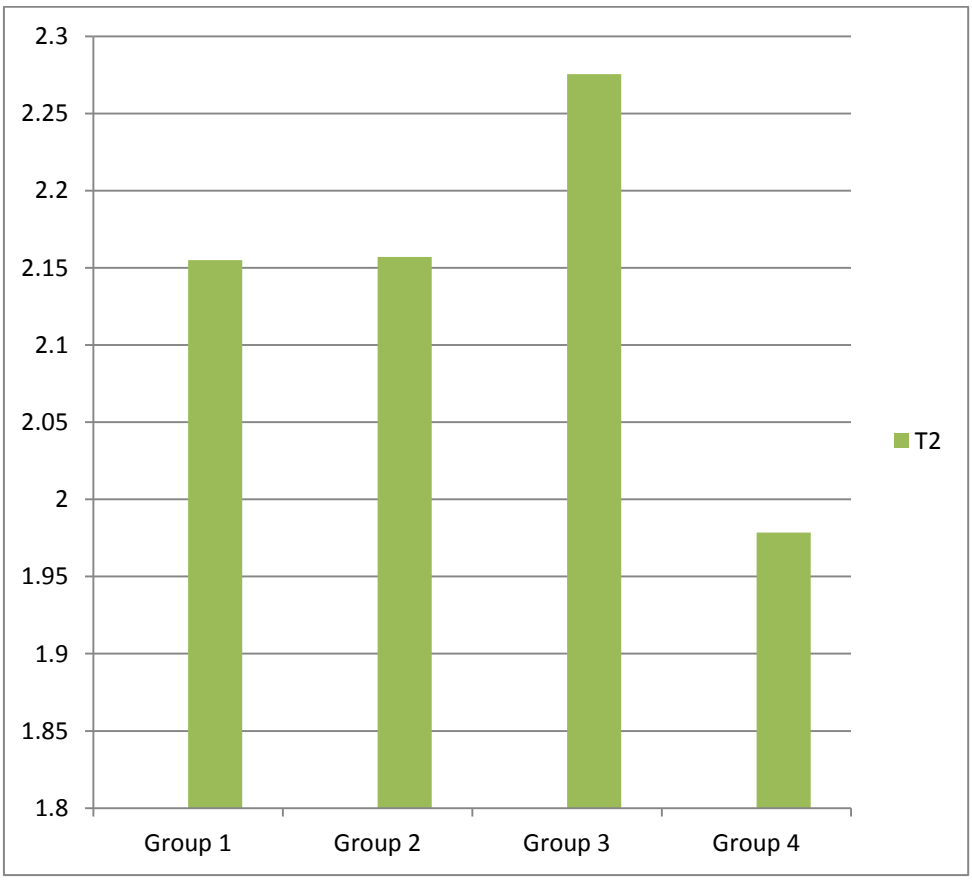
Group 3 ie Yoghurt treated teeth showed the highest amount of remineralization with a mean Ca/P raio of 2.275±0.262 as in Table 2.

Table 2: One way ANOVA for comparison of means between groups at T2.

Time interval	Groups	N	Mean	Std. Deviation	Std. Error	p value
T2	1	20	2.1550	.27144	.06070	.001*
T2	2	20	2.1570	.18860	.04217	
T2	3	20	2.2755	.26255	.05871	
T2	4	20	1.9785	.10796	.02414	

*** p value significant when <0.05**

Bar chart showing the highest mean difference seen in Group 3 at T2



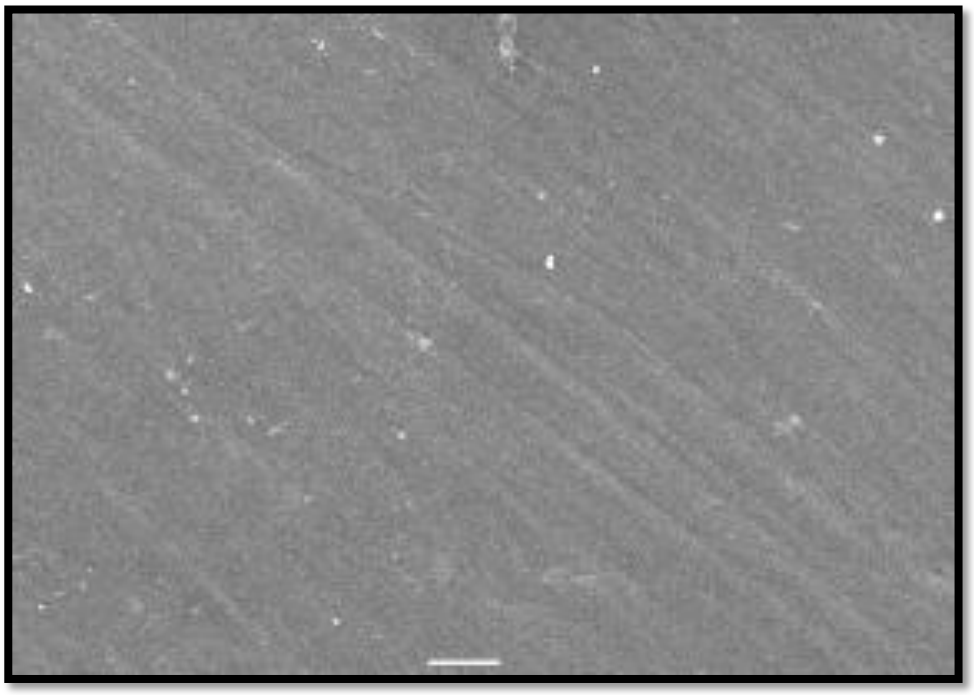
As there was a significant difference in T2- Ca/P ratios among groups, the Post –Hoc Tukey HSD test was done. When Group 1 was compared with the other groups, no statistical significance was seen. Group 2 was compared with the other groups, there was no statistical significance. When Group 3 was compared with the other groups, high statistical significance was found when compared with Group 4(p value <.05) and when Group 4 was compared with the other groups, high statistical significance was found with Group 3 (p value <.05) as in Table 3.

Table 3: Comparisons between groups using Post –Hoc Tukey HSD test at T2.

Dependent variable	(I)Group	(J)Group	Mean Difference (I-J)	Std. Error	p value
T2	Group 1	Group 2	-.00200	.06889	1.000
		Group 3	-.12050	.06889	.306
		Group 4	-.17650	.06889	.059
	Group 2	Group 1	.00200	.06889	1.000
		Group 3	-.11850	.06889	.321
		Group 4	.17850	.06889	.055
	Group 3	Group 1	.12050	.06889	.306
		Group 2	.11850	.06889	.321
		Group 4	.29700*	.06889	.000
	Group 4	Group 1	-.17650	.06889	.059
		Group 2	-.17850	.06889	.055
		Group 3	-.29700*	.06889	.000

*** p value significant when <0.05**

Fig 22: SEM images of enamel surface at T0 (just after bonding) at 2000x magnification



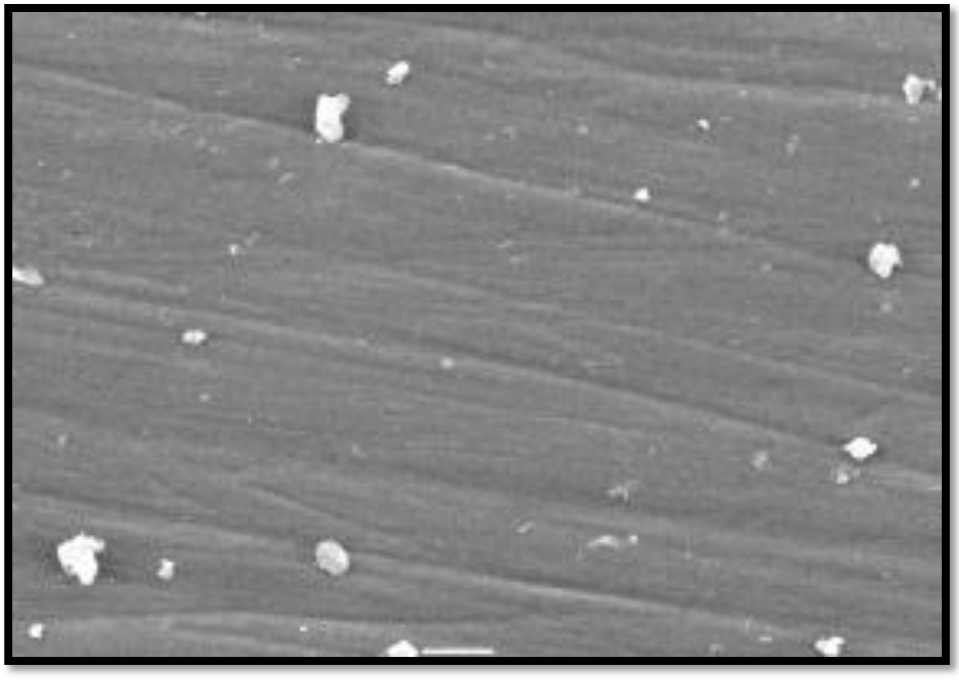
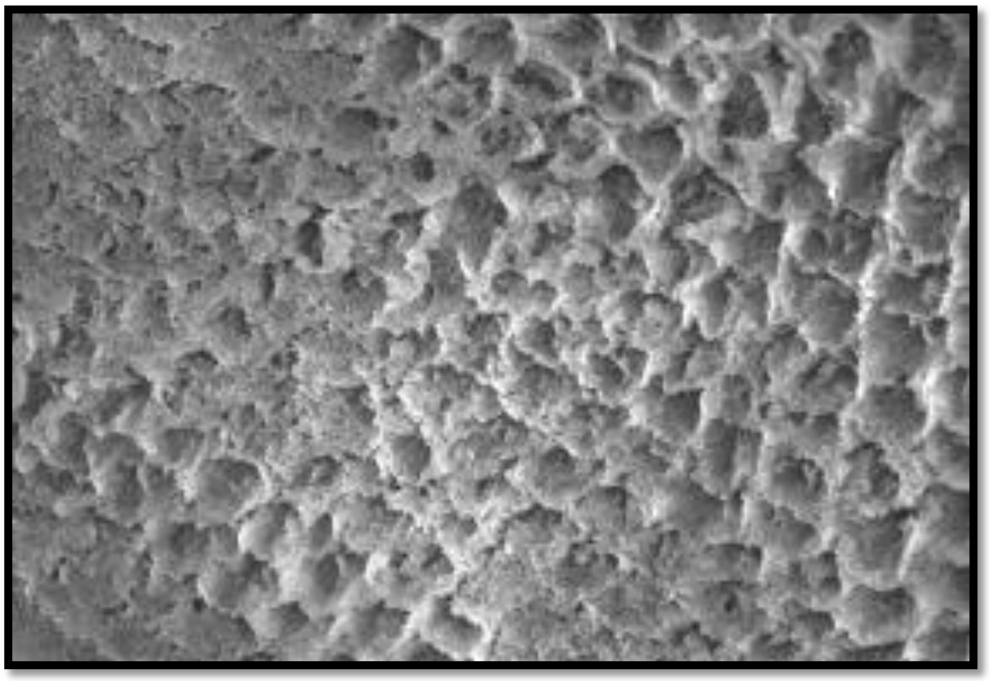


Fig 23: SEM images of enamel surface at T1 (after 48 hours of demineralization) at 2000x magnification



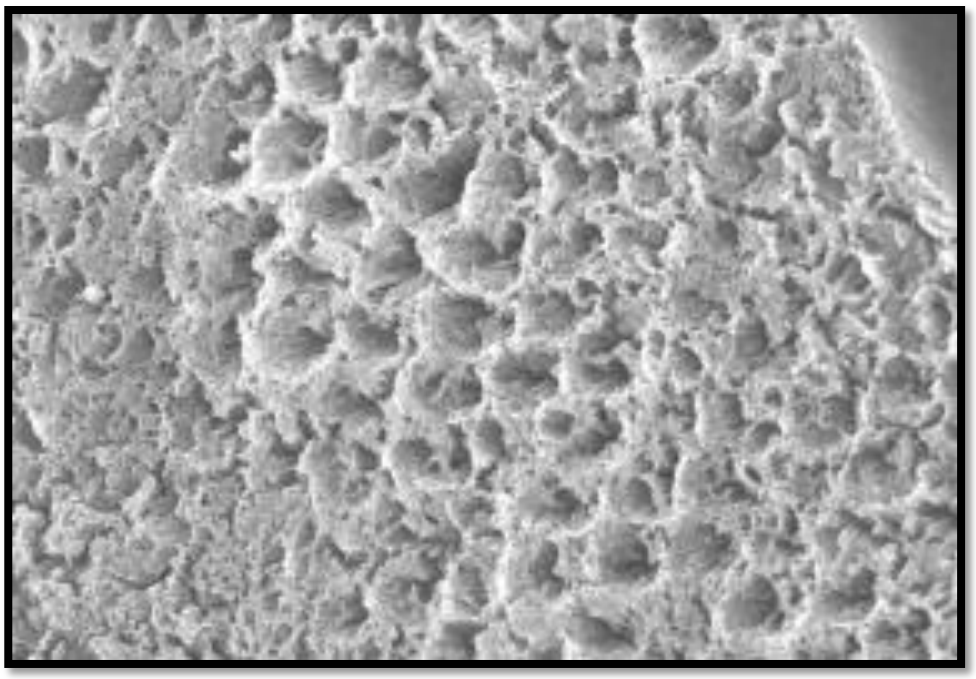
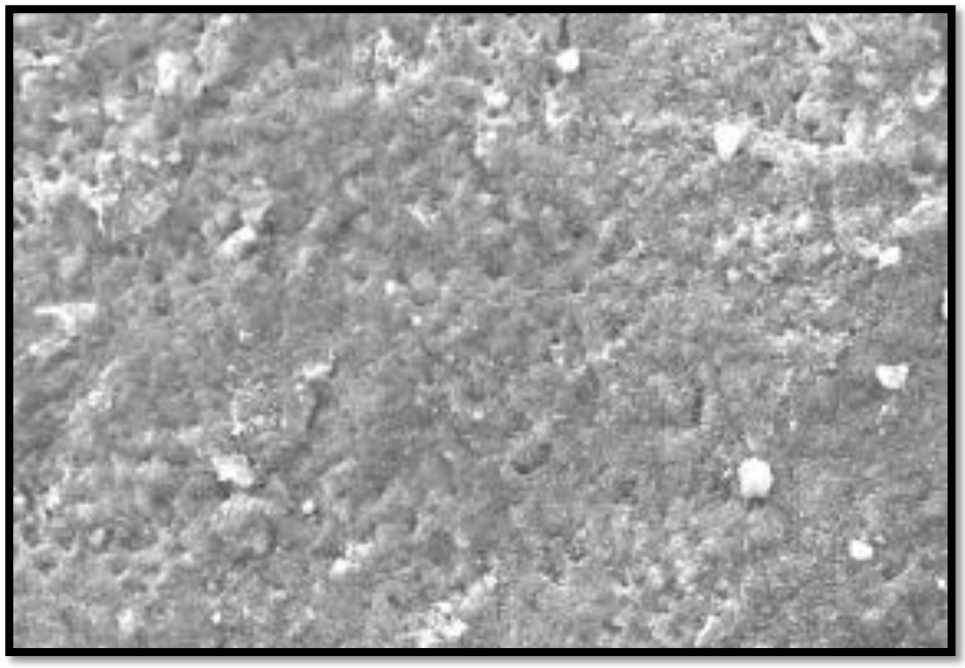


Fig 24: SEM images of Group 1 (Novamin treated samples)

at T2 (after 30 days of remineralisation) at 2000x magnification



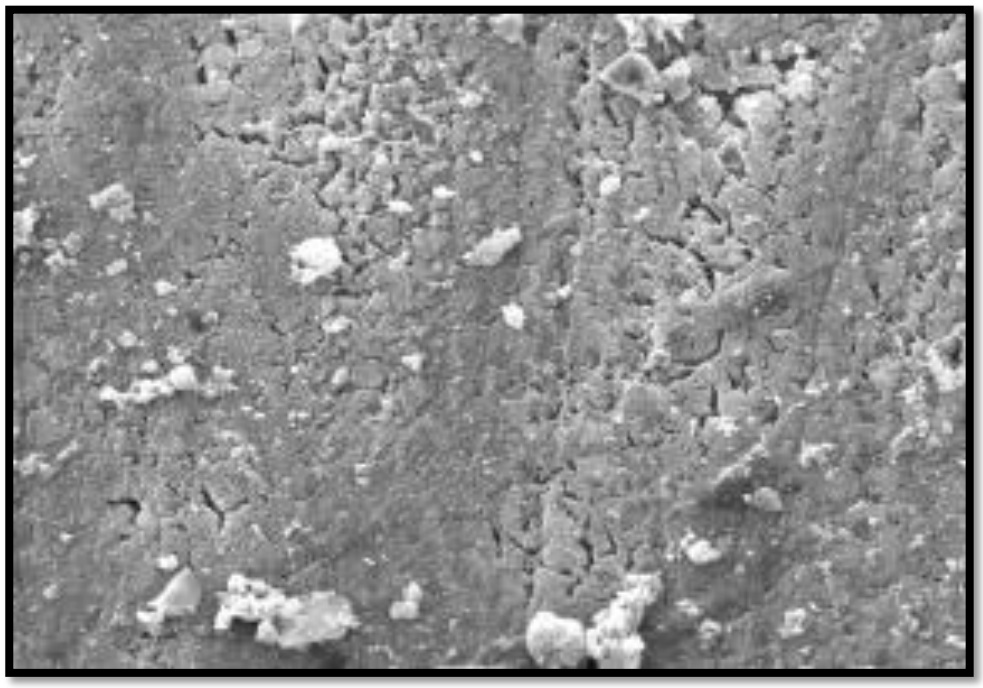
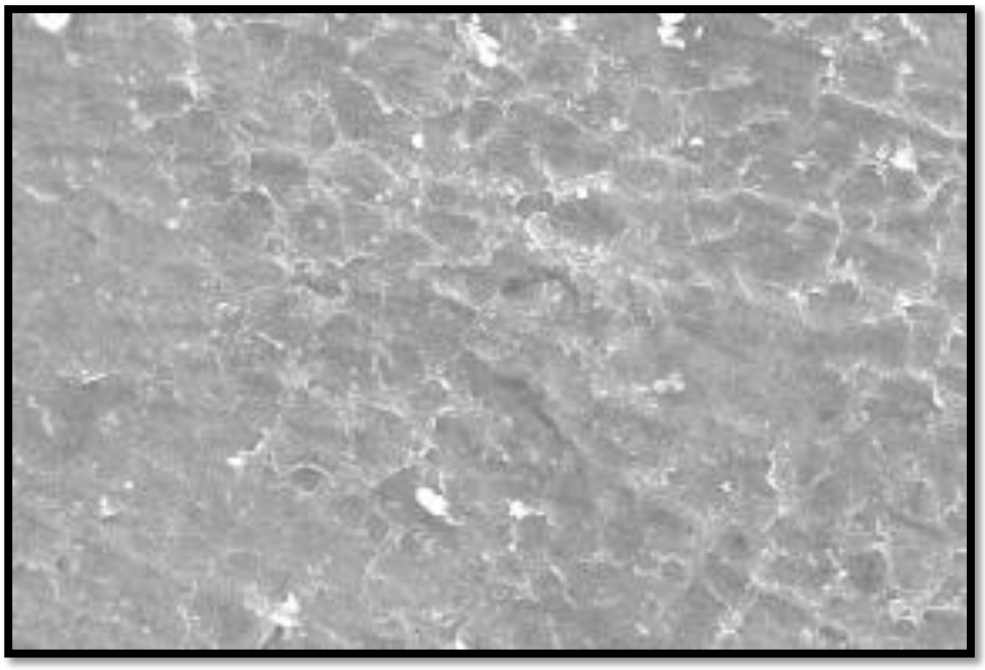


Fig 25: SEM images of Group 2 (Probiotic tooth paste treated samples) at T2 (after 30 days of remineralisation) at 2000x magnification



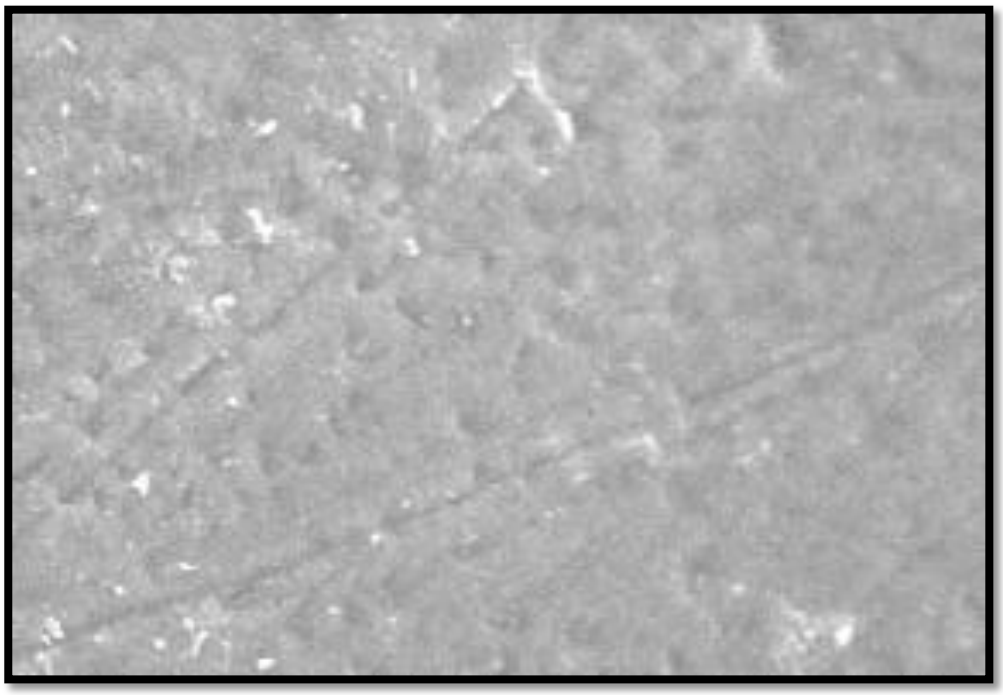
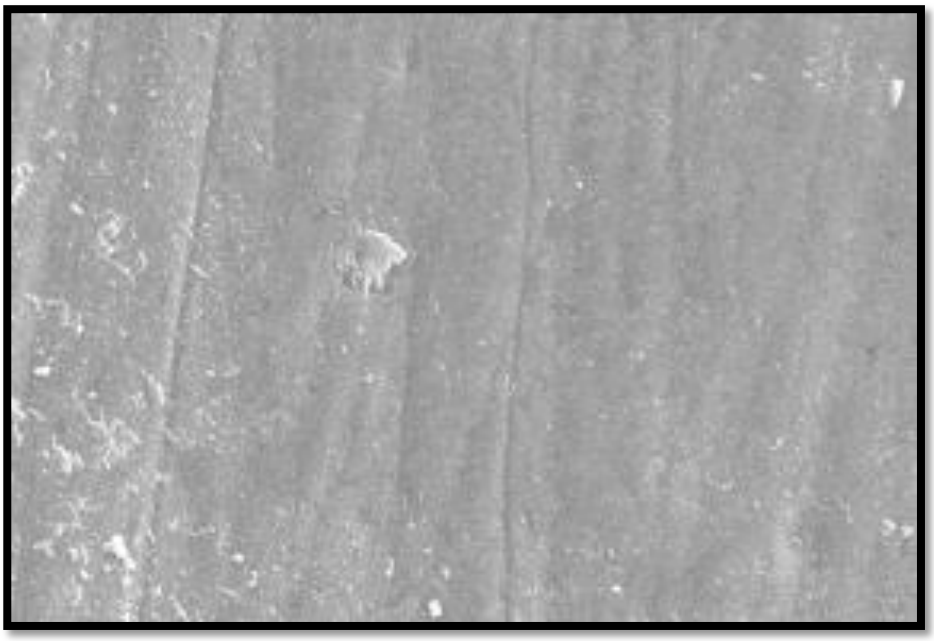


Fig 26: SEM images of Group 3 (Traditional Yoghurt treated samples) at T2(after 30 days of remineralisation) at 2000x magnification



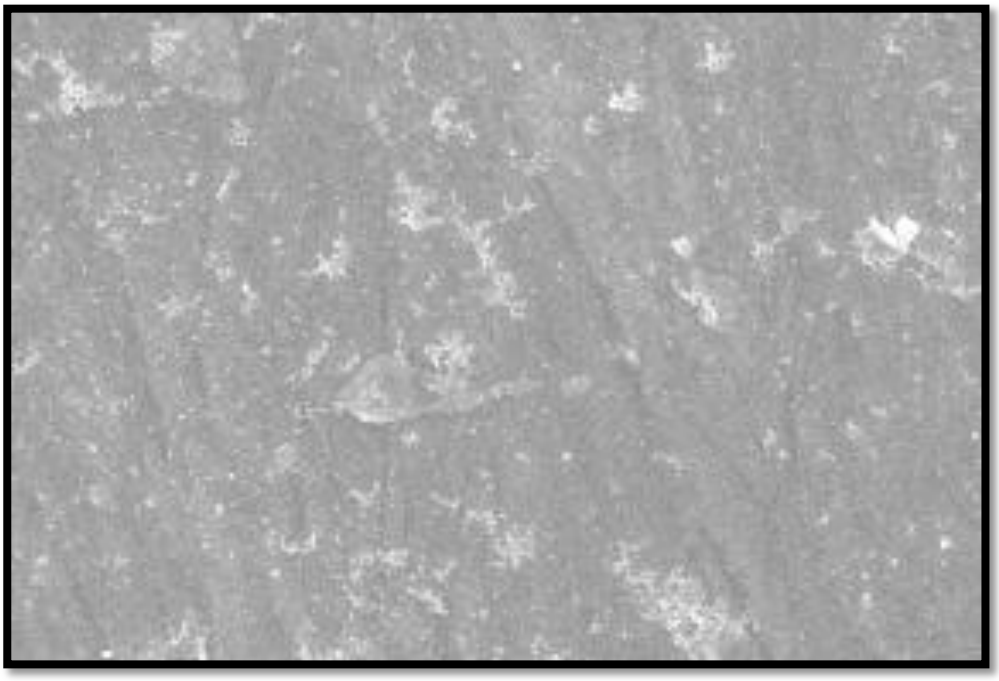
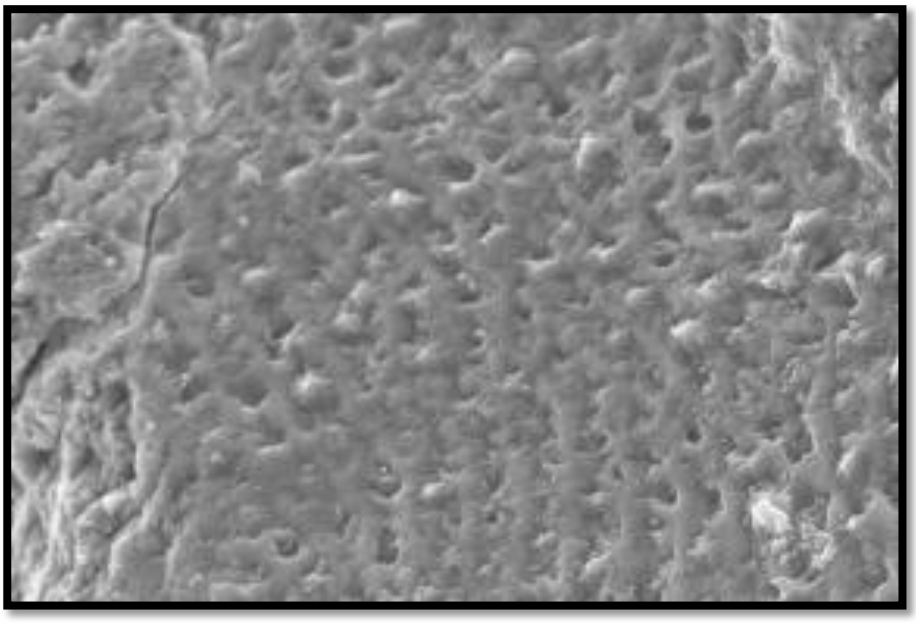
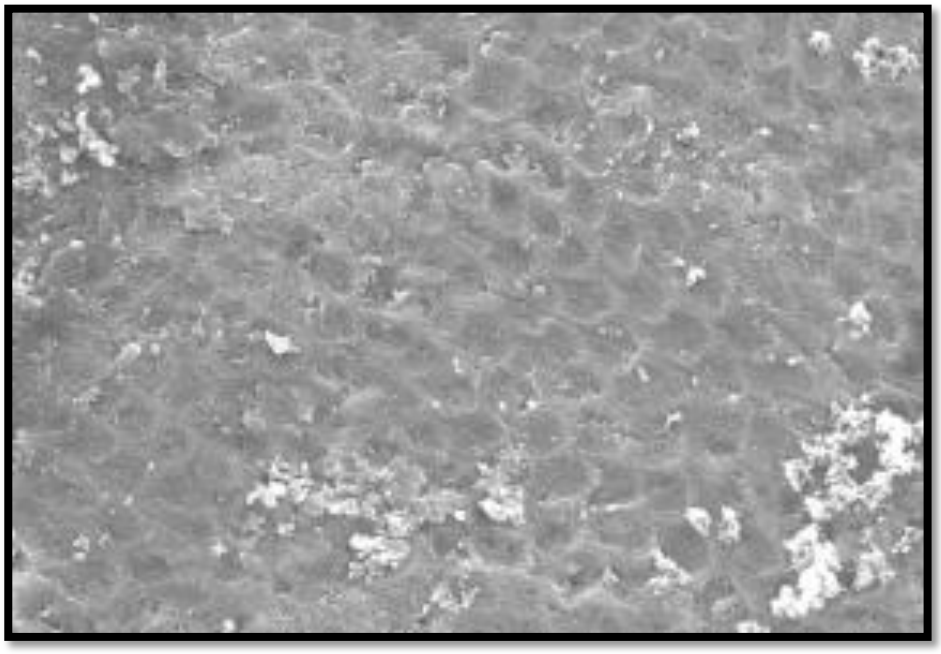


Fig 27: SEM images of Group 4 (Control group)

at T2 (after placing in artificial saliva for 30 days) at 2000x magnification





Scanning Electron microscopic images were taken to evaluate the changes at microscopic level at 2000x magnification at three time intervals.

T0- 0 day just after bonding,

T1- 48 hours of demineralization and

T2 – 30 days post treatment

The untreated samples (ie at T0) showed smooth enamel surface (Fig:22)

The demineralized samples (ie at T1) showed enlarged enamel rods and minute surface elevations. Loss of minerals was evident through the enlarged pore diameter, which measured 3-5µm on an average. The smooth architecture was lost and more of irregularity over the surface was evident (Fig:23)

In the Novamintreated group ie Group 1(at T2), the enamel pores are reduced to the size of around 0.5um, and the remineralisation effect was better than in artificial saliva (control group - 4) (Fig:24)

In the Probiotic treated group ie. Group 2(at T2), remineralization took place as a smooth surface was seen with the enamel pore size reduction to around 0.5-1um. (Fig:25)

In the Yoghurt treated groupie Group 3(at T2), the remineralisation effect was best on comparison of images of other groups. An almost smooth enamel surface was seen with the decrease of the enamel pore size to around 0.25 µm-0.5 µm.(Fig 26)

The control group (at T2) remineralisation with artificial saliva, shows pores with an average diameter of about 2 to 3 µm, the effect of which is minimal.(Fig:27)

DISCUSSION

White spot lesions occur as an iatrogenic side effect of orthodontic treatment[44]. These white spot lesions should be prevented rather than treating and management of these lesions post orthodontically. WSL's occurs when there is an imbalance between the remineralizing and demineralizing cycles. These white spot lesions not only pose an esthetic challenge to the patient but may progress into carious lesions[4]henceforth WSL's are areas of demineralization and hence necessary remineralizing agents should be administerdas an adjuvant to the natural remineralization process.

In the past, fluoridated substitutes have been used for treatment and prevention of these lesions. With the advent of technology and new researches, the bioactive glass which is a biomimetic material has shown greater remineralizing potential than that of the fluoride substitutes. One such bioactive glass is Novamin which helps in the remineralization process of these initial subsurface lesions[(35,38,37,40,39)]

Probiotics in general, favour the growth of useful bacteria and tamper the growth of unfavourable bacteria. Studies have shown that probiotics incorporated in tooth pastes, lozenges etc or through systemic consumption of probiotics, have shown to produce remineralizing effects on the affected enamel[**(23,30,22,45,46)**]

The CPP's are tryptic digestion products of casein, a bovine milk protein. In yoghurt, it is produced partly by the proteolytic activity of the Lactic Acid Bacteria. These CPP's have a natural tendency to remineralize .[26]

There are a variety of remineralizing agents which are commercially available. But the search in need of a easily available and natural product for preventing and remineralising these lesions led to this study. So the study was designed to search for an commonly available remineralising agent . Yoghurt has been proven to produce remineralising effect on artificially produced carious lesion.

The present invitro study was done with 80 extracted human premolars stored in saline until the commencement of study. White spot lesions were created artificially by the Modified pH cycle model by Featherstone [42] which showed clinical correlation with the initial caries development. The remineralizing potential of the Novamin containing paste, probiotic enriched paste and traditional yoghurt was assessed qualitatively using the Scanning Electron Microscope by assessing the surface morphology and to detect the alterations. Demineralised areas usually have a decrease in their Calcium and phosphorus, thereby a decrease in their ratio, and when remineralization occurs, the ratio increases. This ratio assessment was done quantitatively using the Energy dispersive X- ray spectroscopy.

In our study when the groups were compared for their remineralising potential by assessing their Ca/P ratio at T1- (ie after 48 hours of demineralization) and at T2- (ie. 30days post treatment), significant remineralization had taken place in all groups except for the control group – 4 (Table 1).

When Group 1- Novamin treated group when compared at T1 and T2 using Paired T test it showed statistically siginificantremineralization(Table 1) which is in accordance with the study by **Pritam et al**[18] which concluded that Novamincontaining toothpaste showed significant remineralization. When group 1 ieNovamin treated group was compared with the other groups at T2, though there was an increase in the Ca/P ratio but it was not statistically significant according to Post- Hoc Tukey HSD test(Table 3).

Streptococci mutans is the main organism in initiation of carious lesion among the cariogenic microorganisms. Probiotics modifies the microbial flora, create a biofilm, which acts as a protective lining for oral tissues and prevents the adhesion of pathogens. In our study, comparison using the Paired T test at T1 and at T2 showed statistically significance in Group 2 (Table 1) and hence remineralization was effective which is in accordance with **Jubin et al**[23] study which stated that topical application of probiotic tooth paste caused significant decreased in the Streptococcus mutans level in the plaque around orthodontic brackets, which in turn causes reduction in the occurrence of white spot lesions. But when this group was compared with the other

groups at T2 using the Post Hoc Tukey HSD test, it did not show any statistical significance though significant remineraliztion has occurred.

Yoghurt is an excellent source of calcium and phosphorus and has a higher protein content[8]**.** It is one of the readily available, inexpensive common dietary constituent of Indian population. It is demonstrated that the lower the pH of yoghurt as compared with milk it accounts for the favourable ion formation of calcium[47]**.**pH of traditional yoghurt is lower than that of probiotic yoghurt**,**[26] which causes a decrease in the salivary pH causing increased ionic concenteration in the saliva which causes a protective effect and hence the remineralisation process that takes place thereafter. In the present study, traditional yoghurt showed significant remineralisation on comparison using the Paired T test at T1 and T2. On comparison with the other groups at T2 using the Post- Hoc Tukey HSD test, statistically significant remineralization has occurred when compared with the control group and showed an increase mean when compared with the other treated groups which implies that the traditional yoghurt is an potential remineralising agent which is in accordance with **Chanchal et al**[26] and **GF Ferrazzano et al**[41]**.**

The scanning electron microscopic study was done to see the changes at microscopic level . No study was done before to include SEM evaluation and our study was the first one on this subject. The untreated samples showed a smooth enamel surface (Fig 22). The samples after demineralization (ie at T1), showed enlarged rods

and surface elevation and the enamel pore diameter was increased, which shows the loss of minerals and hence significant demineralisation has taken place as in Fig 23

The Novamin treated group ie at T2, the enamel pore size was reduced and hence shows signs of remineralization as seen in Fig:24 and in accordance with the observations of Soares et al [48]

The probiotic treated group, good remineralisation had taken place which is substantiated by the reduction in pore size as seen in Fig:25

The yoghurt treated group the images showed the best remineralisation on comparison with that of the other group images and the pore size was also reduced as seen in Fig:26.

The control group on remineralization with artificial saliva, showed minimal changes and mild decrease in the pore diameter as seen in Fig:27.

Though Group 4 ie yoghurt treated group showed significant remineralizing potential when compared with the control group but when compared with the other

treated groups it did not show any statistical significance on comparison as seen in Table 1.

Although Novamin has been proven to produce remineralisation in many studies[35,38,37,40,39] the present study did not show any statistical significance upon treatment either with the control or the other treated groups.

In the present study the SEM and Ca/P analysis showed yoghurt as a better remineralising agent when compared with that of Novamin and Probiotics which may be attributed to the in vitro conditions of the present study. Further in vivo studies are needed to test the remineralizing capacity of traditional yoghurt which is an easily available indigenious product for effective prevention of WSL's

SUMMARY AND CONLCUSION:

The remineralising potential of a Probiotic tooth paste, Traditional Yoghurt and Novamin containing tooth paste was assessed qualitatively using the Scanning Electron Microscope and quantitatively by Energy Dispersive X-ray Analysis at

T0- 0 days just after bonding

T1- 48 hours of demineralization

T2- 30 days post treatment.

Quantitative assessment was done by assessing the Ca/P ratio of the samples.

The assessment shows that the Traditional Yoghurt has a better remineralising action when compared with the other groups and had statistical significant remineralization when compared with the control group, with the increase in the mean Ca/P ratio after treatment.

Though Novamin and Probiotics showed significant remineralizationon comparing the T1 and T2 values it did not show any statitistical significance when compared with the other groups.

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This study proves that the Traditional Yoghurt has a better remineralising property than the other compared groups. Further invivo studies with larger sample sizes are recommended to test the remineralizing capacity of Traditional Yoghurt.

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